

Appl. No. 09/889,711

Amendment dated January 21, 2004

Reply to Office Action of 09/30/03

**Amendments to the Claims:**

This listing of claims will replace all prior versions and listings of claims in the application:

**CLAIMS**

1. (Currently Amended) A kit comprising:

a) a first polypeptide-cleaving reagent suitable for incorporating in an electrophoretic gel on which at least one polypeptide has been isolated by electrophoresis, said first polypeptide-cleaving reagent being capable of cleaving said polypeptide to produce a partially cleaved polypeptide;

b) at least one hydrophilic membrane ~~suitable for~~ capable of use in transblotting said partially cleaved ~~of polypeptides separated on an~~ from said electrophoretic gel, the membrane having at least one second polypeptide-cleaving reagent immobilised thereon, said second cleaving reagent being capable of further cleavage of said partially cleaved polypeptide to produce polypeptide fragments; and

c) a hydrophobic collection member suitable for receiving thereon said polypeptide fragments from said hydrophilic membrane when ~~of the separated polypeptides~~ transferred thereto by transblotting.

2. (Original) The kit of Claim 1, wherein the hydrophilic membrane and hydrophobic collection member are provided as a pre-formed assembly.

3. (Original) The kit of Claim 1 or 2, wherein the second polypeptide-cleaving reagent is immobilised on the hydrophilic membrane by covalent bonding.

4. (Currently Amended) The kit of Claim 3, wherein the second polypeptide-cleaving reagent is immobilised through an amide linkage formed between (1) the functional groups on the hydrophilic membrane selected from the group consisting of activated carbonyl groups, carboxylic acid groups and carboxylic acid derivative groups capable of reacting with an amino group, and (2) an amino group of the polypeptide-cleaving reagent.

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5. (Original) The kit of Claim 1, 2, 3 or 4, wherein the polypeptide-cleaving reagents are enzymes, which may be the same or different.
6. (Original) The kit of Claim 5, wherein each enzyme comprises a protease.
7. (Original) The kit of Claim 6, wherein the protease comprises trypsin.
8. (Currently Amended) The kit of Claim 1, 2, 3, 4, 5, 6 or 7, further comprising:
  - d) a buffer suitable for at least partially rehydrating said electrophoretic ~~a~~ gel on which at least one polypeptide has been isolated and which has been dehydrated.
9. (Original) The kit of Claim 1, 2, 3, 4, 5, 6, 7 or 8, wherein the hydrophobic collection member is a self-supporting membrane .
10. (Currently Amended) A method of identifying or characterising polypeptides which have been isolated on a gel by electrophoresis, comprising the steps of:
  - a) providing an electrophoretic gel on which at least one polypeptide has been isolated by electrophoresis;
  - b) incorporating a first polypeptide-cleaving reagent in the gel, said cleaving reagent being capable of cleaving said isolated polypeptide contained by said gel to produce a partially cleaved polypeptide;
  - c) providing adjacent to the gel at least one hydrophilic membrane on which is immobilised at least one second polypeptide-cleaving reagent capable of cleaving said partially cleaved polypeptide to produce polypeptide fragments;
  - d) providing a hydrophobic collection member suitable for receiving thereon said fragments of polypeptide transferred thereto from the gel said hydrophilic membrane by transblotting, said hydrophobic ~~layer~~ member being positioned beyond the hydrophilic membrane in a direction of movement of the fragments of polypeptide;

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- e) partially cleaving the isolated polypeptide on the electrophoretic gel by the first polypeptide-cleaving reagent to produce a partially cleaved polypeptide, transblotting the partially cleaved polypeptides from the electrophoretic full-gel, on which the polypeptide or polypeptides were isolated, through the hydrophilic membrane or membranes, under conditions effective to cause it them to be further cleaved into polypeptide fragments by the second polypeptide-cleaving reagent, and transblotting the polypeptide fragments onto the hydrophobic layer collection member; and
- f) identifying or characterising the polypeptide fragments collected on the hydrophobic collection member.

11. (Currently amended) The method of Claim 10, which further comprises:

- g) identifying or characterising the isolated polypeptide from which the polypeptide fragments were derived.

12. (Currently Amended) The method of Claim 10 or 11, wherein the first polypeptide-cleaving reagent is incorporated in the electrophoretic gel by dehydrating the electrophoretic gel and then at least partially rehydrating it with a buffer containing the polypeptide-cleaving reagent.

13. (Original) The method of Claim 10, 11 or 12, wherein the immobilisation of the second polypeptide-cleaving reagent is by covalent bonding thereof to the hydrophilic membrane.

14. (Original) The method of Claim 10, 11, 12 or 13, wherein both the polypeptide-cleaving reagents are enzymes, which may be the same or different.

15. (Original) The method of Claim 14, wherein either or both enzymes cleave the polypeptide in its main chain.

16. (Original) The method of Claim 14, wherein either or both enzymes cleave the polypeptide in a side-chain thereof.

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17. (Original) The method of Claim 14, wherein both enzymes are trypsin and the electroblotting is carried out in a buffer of pH from 8 to 9.

18. (Currently Amended) The method of Claim 10, 11, 12, 13, 14, 15, 16 or 17, wherein the voltage ~~at which the~~ electroblotting is carried out at a voltage which is adjusted to provide a slower than normal transfer through said hydrophilic membrane, so as to extend the residence time of the polypeptide in the proximity of the second cleavage reagent.

19. (Currently Amended) The method of Claim 10, 11, 12, 13, 14, 15, 16, 17 or 18, wherein the electroblotting is carried out under conditions which provide either (1) a discontinuous current from anode to cathode or (2) an alternating current from ~~biased in the~~ anode to cathode direction.

20. (Currently Amended) The method of Claim 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19, wherein the polypeptide fragments are identified by mass spectrometry.

21. (Currently Amended) The method of Claim 20, wherein the membrane is scanned directly by matrix-assisted laser desorption/ionisation time of flight spectrometry and the data obtained therefrom compared with a database, using a computer program, to provide automated polypeptide identification of said isolated polypeptide.